

Henick et al. (2) proposed a method for the analysis of carbonyl compounds which was modified by Ganguly (1). However, certain additional modifications in the procedure were found to improve its usefulness.

Analytical Method

Reagents

- (a) Carbonyl-free benzene: 5 g 2,4-dinitrophenylhydrazine (DNPH) and 1 g trichloroacetic acid (TCA) are added per liter of benzene. The mixture is refluxed for 1 hr and distilled through a Vigreux column. The first and last 50-ml portions are discarded.
- (b) 4.3% (w/v) TCA in carbonyl-free benzene (TCA solution)
- (c) 0.05% (w/v) DNPH in carbonyl-free benzene (DNPH solution)
- (d) 80% (v/v) aqueous ethanol (80% ethanol)
- (e) 4% (w/v) KOH(CP) in 80% ethanol (alc. KOH)
This solution is aged for at least ten days.
- (f) Heptaldehyde: obtained from Eastman Organic Chemicals.
- (g) $K_2Cr_2O_7$ solution.
- (h) Heptaldehyde 2,4-dinitrophenylhydrazone (heptaldehyde DNPH): Prepared by the method of Shriner et al. (7). After recrystallization, it is retained on a sintered glass filter and washed free of hydrazine by the method of Iddles et al. (3). It is dried overnight in a desiccator and the melting point is determined as a check of its purity.

Experimental Procedure

Permanent color blank. To avoid errors due to fading of the color in the reagent blank during a series of analyses, a permanent color blank was prepared. Three milliliters of TCA, 5 ml DNPH, and the same quantity of solvent as needed for the determination of carbonyl compounds were transferred to a 50-ml round-bottom flask fitted with a standard taper neck and incubated for exactly 30 min in a 60°C water bath to form the DNPH derivatives. The solvents were evaporated completely at 30-40°C in a rotary evaporator under a partial vacuum produced by a water aspirator, and the color was developed by adding 10 ml alc. KOH and diluting to 50 ml with 80% ethanol. Twelve minutes after the addition of alc. KOH, photo-

metric readings were made at 435 m μ on a Coleman Model 14 Spectrophotometer against a mixture of 40 ml 80% ethanol and 10 ml alc. KOH. A $K_2Cr_2O_7$ solution having the same per cent transmission at 435 m μ is prepared for each series of determinations as a permanent color blank.

Determination of the molar absorptivity. A plot of log per cent transmission or absorbance vs. concentration was found to be linear over the range of 0-2 μ moles/50 ml. However, it was difficult to obtain a valid standard curve, because of impurities in the available heptaldehyde. Therefore, it was found more desirable to calculate the heptaldehyde concentration on the basis of the molar absorptivity of its 2,4-dinitrophenylhydrazone. A series of known quantities ranging between 0.1 and 0.6 μ moles of the heptaldehyde DNPH in benzene was used to determine the molar absorptivity. The procedure is the same as for the reagent blank, described under the preparation of the permanent color blank, except that no DNPH is added and the incubation step is omitted. A new molar absorptivity is determined whenever a new alc. KOH is prepared. During the course of this study, two values were determined and found to be 24,154 and 23,646, showing good agreement with the results of Jones et al. (4).

Analytical procedure. The analytical procedure is identical to that for the reagent blank, described under the preparation of the permanent color blank, except that the photometric readings are taken with the permanent color blank set at 100% transmission. The quantity of solvent containing the carbonyls may vary from 1-20 ml and is chosen to give a per cent transmission in the region of minimum error. The amounts of carbonyls expressed as micromoles of heptaldehyde are calculated from the formula $C = A/Ed \times 10^6$, where C is the quantity of carbonyls present, A is the absorbance, E is the molar absorptivity, and d is the cell thickness.

Establishment of Conditions for the Test

Time between color development and measurement of transmission. Since the color of the 2,4-dinitrophenylhydrazones of aldehydes is not stable, the photometric determination must be made at a precisely defined time following color development. To determine the most appropriate time interval between color development and measurement, a solution of heptaldehyde in hexane was prepared and the color developed as in the analytical procedure. Transmission readings were determined at equal time intervals. The color faded linearly with time from 3 to 24 min and, therefore, 12 min was selected as a convenient time interval. The linear regres-

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sion coefficient for the relationship is 0.614% transmission per minute.

Reaction time. To determine the degree of completion of the DNPH-carbonyl reaction, a standard solution of 314.9 μM /l heptaldehyde in hexane was prepared and the test performed on 10-ml aliquots, with incubation times of 30, 60, and 90 min, respectively. An analysis of variance revealed no significant differences, indicating that the reaction is complete in 30 min.

Volume and type of solvent. In the test as originally proposed, only 1 ml of solvent containing the carbonyl compounds was used. To determine if it was possible to use larger volumes of solvent, different volumes of hexane containing the same amount of heptaldehyde were analyzed according to the procedure. The evaporation of the solvent was considered to be complete when the last visible traces of solvent disappeared. Experimental evidence was obtained and an analysis of variance confirmed that no significant error was introduced by continuing the evaporation 4 min past the visual end point. An analysis of variance of these results revealed no significant differences in the tests when initial volumes of 1, 5, 10, 15, and 20 ml hexane were used.

It was also verified that the test may be performed with several solvents, such as petroleum ether, hexane, carbon tetrachloride, benzene, and dichloroethylene, the only requirement being that the solvent be carbonyl-free, or that its carbonyl content remain constant. Ethyl ether is an unsuitable solvent, in that it produces interfering substances even during the short period of time required for the test.

Reproducibility. An indication of the reproducibility of the test was obtained from an experiment in which four cans of evaporated milk of identical history were subjected to a solvent partitioning technique according to Patton (6) and modified by Muck et al. (5). The carbonyl test was run in duplicate on 10-ml aliquots of the extracts obtained from each can. As shown in Table 1, the standard deviation for the solvent partitioning procedure was $\pm 0.0836 \mu\text{M}$ per ml of recovered solvent, while that for the analytical procedure was $\pm 0.0328 \mu\text{M}$ per ml of recovered solvent.

² Present address: Dean Foods Company, Rockford, Illinois.

TABLE 1
Reproducibility of the carbonyl test

Sample	Volume of hexane recovered	Mean ^a A	Mean carbonyls per ml of recovered solvent
	(ml)		($\mu\text{moles/ml}$)
1	70	0.221	0.93
2	69	0.196	0.81
3	72	0.191	0.81
4	74	0.196	0.83

^a Tests were made on 10-ml aliquots of solvents recovered.

G. A. MUCK²
N. R. SUNDARARAJAN
J. TOBIAS
and
R. McL. WHITNEY
Department of Food Science
University of Illinois, Urbana

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